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THE REQUIREMENTS OF FATTY ACIDS, CHOLESTEROLS AND PHOSPHOLIPIDS FOR FISHES AND PRAWNS

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Key words

Fatty acid, cholesterol, phospholipid, fish and prawn

Certain fatty acids and fatty substances like cholesterol and phospholipids can be biosynthesized by fishes and prawns on their own, but some cannot be synthesized, or the amount synthesized falls far short of the amount required by fishes and prawns during their growing stage. The deficiency that arises as a result of this seriously affects the survival and growth of juvenile fishes and prawns. One way to better satisfy the requirement of fishes and prawns for fatty acids, cholesterol and phospholipids is to raise the level of nutrition from external sources. Hence, an understanding of the physiological functions, deficiency diseases and amount required of fatty acids, cholesterol and phospholipids in the lives and activities of fishes and prawns is most helpful towards formulating a standard diet for fishes and prawns and improving their survival and growth rates.

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Physiological Functions

(1) Essential Fatty Acids

Fishes and prawns derive their fatty acids from hydrolyzation of fats and also from biosynthesis: for example, the beta-oxidation of fat and the production of acetyl-Co A, a precursor of biosynthesized fatty acid from the catabolism of protein and sugar. However, Mead *et al.* (1960) proved by means of a tagging experiment that *Tilapia mossambica* cannot synthesize (1-^{14}C) acetic acid into polyunsaturated fatty acids (PUFA). G. Burr and M. Burr (1929, 1930) were the first to determine that there must be a special unsaturated fatty acid in the diet of animals which cannot be synthesized by the animals themselves and has to be derived from food. Subsequent to this, Aaes-Jorgensen (1961), Alfin-Slater *et al.* (1968) and Holman (1968) published their discoveries of the same type of fatty acid in homeotherms and classified oleic acid, linoleic acid, linolenic acid and arachidonic acid as essential fatty acids (EFA) for animals. The modern trend is to classify all unsaturated fatty acids as essential fatty acids for animals. These are fatty acids that must be derived from food.

As for such poikilotherms as fish and prawns, the EFA required for their growth and survival varies from one kind to the other. Judging from the fatty acid composition in marine and freshwater fish, the n-6 series (the carbon nearest the unsaturated bond in methyl) of freshwater fish is higher than that of marine fish, and the average n-6/n-3 ratio of freshwater fish is higher than that of marine fish. *Tilapia zillii*, which is reared in freshwater, requires only the n-6 fatty acid (Kanazawa *et al.*, 1980). The difference in EFA in marine and freshwater fish is due to differences in the fatty acid composition of their food or to characteristics developed for adapting to their environment. Cold-water fish require more n-3 than n-6. The rainbow trout (*Salmo gairdneri*) requires a large amount of n-3. It is believed that these highly unsaturated EFA's are essential for maintaining the pliability and permeability of the phospholipid membrane in low temperatures (Castell *et al.*, 1972 a, b, c). Warm-water fish require a mixture of n-3 and n-6. Fish require more n-3 than n-6 during the reproductive stage, particularly the female fish. This is because the ovaries and ovules require more n-3 unsaturated fatty acids for the larval fish that will be hatched. Juvenile fish also require n-3 unsaturated fatty

acids for their growth (Kanazawa, 1985). The data in Table 1 show that when feeding on a diet lacking in EPA, the rainbow trout and red sea bream (*Chrysophrys major*) have lower spawning and hatching rates than the control group. Furthermore, the eggs and larval fish of the red sea bream have a high deformity rate. Shimma *et al.* (1977) proved that the amount of 22:6n-3 in the diet has a major effect on the spawning rate of the carp. Middleditch *et al.* (1980) discovered that unless there are 20:5n-3 and 22:6n-3 in the diet, the white prawn (*Penaeus setiferus*) will not spawn. Morris (1973) proved that juvenile marine crustaceans have a higher EFA content than mature individuals of the same species. Moreover, 18:2n-6 and 20:5n-3 in PUFA are the precursors of prostaglandin in fish. Other hormones also contain EFA.

(2) Cholesterol

Cholesterol is the most typical sterol of its kind. It is the precursor of sex hormones, pararenocortins [?], bile and other essential substances in the body which sustain life. Although fish can synthesize sterols from acetic acid and mevalonic acid (Kayama *et al.*, 1965; Hazel *et al.* 1979), crustaceans do not have this ability. The change from cholesterol to sex hormones and pararenocortins [?] in the bodies of crustaceans has already been confirmed in the Japanese lobster (*Pamulrus japonica*) (Kanazawa *et al.*, 1979d). It was also reported that after injecting radioactive cholesterol into the bodies of the Japanese prawn (*Penaeus japonica*), cholesterol was found to accumulate in the hypodermal cells. It was thus deduced that cholesterol is the substance for synthesizing the decrustation hormone. Fish and prawn can also make use of other sterols aside from cholesterol, such as ergosterol from yeast and stigmasterol and sitosterol from the soybean. The effect, however, is not as good as that of cholesterol (Table 2).

(3) Phospholipids

Phospholipids are also commonly known as lecithin. This includes soy lecithin, choline phosphatide, lipositol, cephalin, sterol lipids, glycolipin and phosphatidate. Their chemical components are phosphorus, nitrogen, choline, inositol, sterols, oil-containing substances, and ash. In view of the complexity of their chemical composition, phospholipids are highly nutritious and possess the nutritional value of EFA, vitamins (choline and inositol) and sterols.

Phospholipids are essential substances in the structure of the organism and play an important part in the formation of the biomembrane cell and subsidiary cell. Phospholipids are the most important transportation lipids in the body fluids of crustaceans. EFA, consisting of phospholipids, takes part in the transportation of other lipids (such as sterols) (Mead *et al.*, 1960; Aaes *et al.*, 1967; Sire *et al.*, 1981). They help in the dissolution and absorption of fats and will also promote the absorption of vitamin A and carotene. Phospholipids participate in sodium and potassium ion activity to stimulate certain neurological tissues (Tanaka *et al.*, 1964; Kimelbery *et al.*, 1972). As phospholipids play a part in the transportation and absorption of cholesterol, they also affect the decrustation process of crustaceans. From practical experience with diets for aquatic animals, it has been found that the addition of phospholipids to the diet can defer the dissolution and loss of soluble nutrients, thus enabling the food to increase in lubricity for better particle formation. The addition of phospholipids to the diets of larval Japanese prawns can prevent a high death rate. The addition of lecithin from the lipids of short-necked clams (*Tapes sp.)* to the semi-refined diet of Japanese prawns is effective in promoting growth (Kanazawa *et al.*, 1979d).

During the period of rapid growth after hatching, large quantities of phospholipids are needed for cell formation. When the biosynthesis of phospholipids cannot fully satisfy the needs of the larval fish, it is necessary to add phospholipids to their diets. Kanazawa (1985) reported that the addition of phospholipids to microparticulate diets can significantly improve the growth and survival rates of larval fish. Table 3 shows the composition of diets for the larval red sea bream. This diet was used for feeding red sea bream 10 to 20 days after hatching. A diet maintaining cod-liver oil at 3% and soy lecithin at 5%, as compared to a diet containing only 3% or 6% cod-liver oil, can more effectively promote the growth and survival of the fish. By monitoring the growth and survival rate of the fish, it was found that diets with the addition of phospholipids are comparable to diets to which live rotifers have been added.

Kanazawa (1985) had previously fed ayu (*Plecoglossus altivelis*) on a diet similar to that used for the red sea bream. When 3% egg lecithin, tuna lecithin or soy lecithin was added to the diet, the growth and survival rates of the fish were improved. The tuna lecithin was also found to be more nutritious than soy lecithin or egg lecithin. Phospholipids are required in different amounts for

survival and growth. Phosphatidylinositol and lecithin are both effective for survival and growth, but phosphatidylethanolamine and phosphatidylcholine have little or no effect on improving the survival rate. The addition of phospholipids will also reduce the incidence of malformation in fish. For example, the incidence of scoliosis is 63% in larval fish feeding on rotifers, artemias and commercial diets, whereas for larval fish feeding on microparticulate diets to which soy lecithin or egg lecithin have been added, the incidence of scoliosis is only 5% (Table 4).

Deficiency Diseases

EFA-deficiency diseases are abnormal growth phenomena that appear in fish and prawns that feed on diets devoid of fat or lacking in EFA. EFA-deficiency symptoms appear only after the fish have fed on EFA-deficient diets for a long period of time. Castell *et al.* (1972 a, b, c) put cold-water juvenile rainbow trout on a fat-deficient diet for one month to exhaust all supply of EFA in the fishes' bodies. They were then started on various experimental diets lacking in fatty acids. EFA-deficiency diseases appeared in the form of slow growth, reduced diet efficiency, higher death rates, increased amount of water in the muscles, loss of body colour and serious fin rot (particularly in the tail fin). The loss of lipid function causes a change in membrane permeability. For example, when immersed in an isotonic cane sugar solution, the chondriosomes of an excised hepatocyte will increase in lipid rate, the liver fat will change in character, the respiration rate of the liver homogenate will increase, the hemoglobin level will decline, and the size of the red blood cells will decrease. Feeding rainbow trout an n-3-deficient diet (but with n-6 present) will also cause fainting or shock syndrome to appear. When unsaturated fatty acids like n-6 or n-3 are lacking in the diet, the amount of 20:3n-9 synthesized by the rainbow trout will increase.

Warm-water fish such as the carp and eel can also suffer from EFA-deficiency diseases. Takeuchi *et al.* (1977) put some carp on a fat-deficient diet for 6 weeks and then fed them on an experimental EPA-deficient diet for either 11 or 14 weeks. This resulted in a lowered growth rate and diet efficiency among the fish. The death rate increased and the quantity of 20:3n-9 in the fishes' bodies increased. The EFA-deficiency diseases observed in the eel are the same as those in the carp (Takeuchi *et al.*, 1980).

The ayu and red sea bream require n-3 PUFA, such as 20:5n-3 and 22:6n-3, in both the juvenile and mature stages. In Japan, rotifers [*lit.*, the creased arm-tail rotifer] which had originally been fed with chlorella (which contain a high level of n-3) were fed instead with bread yeast. These rotifers were in turn used to feed juvenile fish. This resulted in a high death rate among the juvenile fish. Red sea bream larvae that were fed with rotifers and artemias lacking n-3 PUFA suffered from poorly developed air bladders, scoliosis and other deformities (Watanabe, 1982; Cka *et al.*, 1980).

Castell *et al.*, (1975) found from their study that the American lobster, when deprived of EFA, will not only suffer a reduction in growth rate and diet efficiency but will also experience a reduction in the number of hemocytes, the amount of hematocyanin and the percentage of edible meat. Furthermore, the period of time between two episodes of decrustation is lengthened and the amount of meat gain decreases. Kanazawa *et al.* (1979c) discovered that when Japanese prawns are fed with EFA-deficient diets, the amount of 20:3n-9 in the prawns' bodies is very low, whereas the amount of 18:1n-9 increases. He therefore decided to use the ratio of (18:0 + 18:1n-9)/20:5n-3 or (18:0 + 18:1n-9)/22:6n-3 as an indicator of EFA deficiency in the prawns' bodies. The higher the ratio, the more serious the deficiency.

The lack of sterols in the diet of crustaceans will result in a very high death rate within a short time. D'Abramo *et al.* (1981) pointed out that the lack of lecithin in the diet of lobsters will prevent complete decrustation and lead to a high death rate.

Amount Required

Studies on the amount of fat required by fish and prawns can hardly satisfy the demand for research on the kind of fat required. Even when the amount of fat is the same, differences in the nature of the fat will produce different results in cultivation. Much has been reported abroad on the amount of EFA required by cultivated fish and crustaceans (Table 5). While there are both qualitative and quantitative conclusions for some species in regard to EFA requirement, others have only qualitative conclusions. There is yet no conclusion on the EFA requirement of the channel catfish (Gatlin *et al.*, 1982). Generally speaking, the EFA requirement of fish and crustaceans is about 0.5-2% of the diet.

When studying the amount of EFA required by fish and prawns, it is necessary to pay attention to the relationship between the synthesis, conversion and inhibition of fatty acids. When fed with 18:3n-3 for a long period of time, freshwater fish like the carp, trout, eel and ayu experience an increase in the amount of 20:5n-3 and 22:6n-6 in the body. This ability, however, is lacking in marine fish like the flounder and red sea bream. Kanazawa (1985) called this ability of fish to transform 18:3n-3 into the longer-chained n-3 series "relative biological conversion ability" (with rainbow trout having an RBCA of 100). RBCA = 36 for the ayu, 20 for the eel, 15 for the red sea bream, 13 for the red-finned oriental globefish (*Spheroides rubripes*) , and 7 for the grouper (*Epinephelus sp.*) . The short-chained n-3 can be synthesized into the long-chained n-3 but not into n-6 (Fuji *et al.*, 1976).

The rainbow trout can convert 18:2n-6 and 18:3n-3 to fatty acids in the same series as C 20 or C 22. The red flounder (*Pleuronectes platessa*) can synthesize C 12 and C 14 fatty acids into a C 18 saturated or single double-bonded unsaturated fatty acid, but it cannot synthesize 20:3n-9. Fed with 18:2n-6 and 18:3n-3, it cannot synthesize 20:4n-6, 20: 5n-3 or 22:6n-3 either. *Scophthalmus maximus* cannot convert the 18:2n-6 in its diet to 20:4n-6 or synthesize 18:1n-9 into 20:3n-9 (Owen *et al.*, 1972).

The mutually inhibiting ability of EFA in fish occurs in this order: n-3›n-6›n-9 (Mobrhauer *et al.*, 1963). This is confirmed in the experiment on the channel catfish. Large amounts of 18:2n-6 and 18:3n-3 in the diet will reduce the amount of synthesis of n-9 fatty acids and lower the rate of metabolism. This is the result of fatty acids inhibiting one another (Holman *et al.*, 1963; Garcia *et al.*, 1965).

Research on EFA in fish had a late start in China. CHEN Shaolian *et al.* (1983), LI Danqiu (1989), Ye Xinrong *et al.*(1990) studied saturated and unsaturated fatty acid composition in the fats of fish and prawns. Progress made in these testing techniques will provide methods for further research on the EFA requirements of cultivated fish and prawns.

As regards the need of crustaceans for cholesterol and phospholipids, Kanazawa *et al.* (1971) reported that Japanese prawns require 0.5% cholesterol in their diet. This is the same as the

amount of cholesterol needed by *Penaeus monodon* , as determined by WU Chunheng (1986). Deshimara *et al.* (1974), however, maintained that Japanese prawns require about 2% cholesterol in their diet. Research on the American lobster shows that 0.5% cholesterol is required (Castell *et al.*, 1975). The lecithin added to the diet of prawns should be 1-2%, while 7-8% soy lecithin should be added to the semi-refined diet of juvenile American lobsters which is basically composed of casein and albumen (Conklin *et al*, 1980). The addition of 5% soy lecithin to the diet of larval ayu, as compared to the addition of only 1% or 3% soy lecithin, can promote their growth and survival rates (Kanazawa, 1985).

Takeuchi (1979) discovered that feeding the rainbow trout with 14% or even more 18:3n-3 or other n-3 PUFA will result in a slower growth rate and lower diet efficiency. Diets with high 18:2n-6 or 18:3n-3 content are also unfavorable to the channel catfish (Stickney *et al.*, 1971, 1972). Over 5% cholesterol in the diet of Japanese prawns will impede their growth.

The amount of fat and EFA required by fish and prawns not only varies according to the species but is is also affected by temperature, salinity and other environmental factors as well as biological and ecological factors such as age, season and moulting cycle. Research data show that the composition of fatty acids and phospholipids in fish is greatly affected by temperature (Reiser *et al.*, 1963; Farkas *et al.*, 1980). Salinity can also affect the amount required. Rainbow trout taken from the same source were placed simultaneously in seawater and freshwater. The experiment showed that the fish that were placed in seawater experienced a loss of EFA at a much faster rate (Lall *et al.*, 1979). Thus, at the same time as the amount of fat required for a certain cultivated species is reported, the biological characteristics of the species and ecological conditions of the experiment should also be indicated.

Table 1: Effect of Essential Fatty Acids on Spawn of Two Species of Fish (from Watanabe, 1982)

	Quantity of Spawn	Hatching Rate (%)	Deformity in Eggs and Larval Fish (%)
Rainbow trout			
Control group	2375	70.0	- - -
EPA-deficient	1429	46.4	- - -
Red sea bream			
Control group	1.74×10^6	93.9	1.8
EPA-deficient	1.17×10^6	0.9	90.0

Table 2: The effect of Various Dietary Sterols on Growth and Survival Rates in Kuruma Shrimp (*Penaeus japonica*) (from Kanazawa *et al.*, 1971)

Sterol Added (0.5g/100g feed)	Survival Rate(%)	Growth Rate (%)
Cholesterol	86-95	56-98
Ergosterol	87-94	48-79
Stigamsterol	83-96	56-67
Beta-sitosterol	83-92	29-59

Table 3: Composition (%) of Microparticulate Diets for the Larval Red Sea Bream (*Chrysophrys major*) (from Kanazawa, 1985)

Composition	Diet 1	Diet 2	Diet 3
Casein	52	52	52
Gelatin	11	11	11
Dextrin	12	12	12
Amino acid mixture (1)	5	5	5
Vitmain mixture (2)	3	3	3
Mineral mixture (3)	8	8	8
Oleic acid	6	3	1
Cod-liver oil	3	6	3
Soy lecithin	0	0	5
Maize protein	62.5	62.5	62.5

Note: (1) Amino acid mixture (gm/100 gm dry feed, except for maize protein): phenylalanine 0.4; arginine 0.9; cystine 0.5; tryptophan 0.2; histidine 0.2; alanine 0.9; asparaginic acid 0.7; lysine 0.4; valine 0.5; glycocoll 0.3. (2) from Halver (1957). (3) US Pharmaceutical Encyclopedia XII.

Table 4: Effect of Lecithin on the Incidence of Malformation in Ayu (*Plecoglossus altivelis*) larvae (from Kanazawa, 1985)

Incidence of Malformation	Rotifers+Commecial Diet	Rotifers+Micro-particulate Diet with Lecithin
Lion's head	1	0
Protruding chest	1	0
Double jaw	8	3
Scoliosis	10	0
Double tail	35	0
Incidence rate (%)	63	5

Table 5: Essential Fatty Acid Requirements among Fish and Shellfish

Type	Testing Temperature (°C)	Feeding Test (weeks)	Amount Required	Source
Carp	20-25	11-14	Mixture of 1% 18:2n-6 & 1% 18:2n-3 superior to a single kind. PUFA 20:5n-3 and 22:6n-3 more effective than the above 2.	Takeuchi *et al*, 1977
Eel	25	10	Mixture of 0.5% 18:2n-6 & 5% 18:3n-3, or mixture of 0.5% 20:5n-3 & 20:6n-3.	Takeuchi *et al*, 1980
Tilapia zillii	27-28	4	1% 18:2n-6 or 1% 20:4n-6 superior to 18:3n-3	Kanazawa *et al*, 1980
Channel catfish	26-33		High 18:2n-6 or 18:3n-3 content (10% safflower oil or 10% linseed oil) will retard growth, others unknown	Stickney *et al*, 1971,1972; Murray *et al*, 1977
	20-23	10		
	23-28	56		
Yellowtail	23-28	6	Fish oil superior to cereal oil	Tsukahara *et al.*,1967
Ayu	--	--	PUFA (20:5 and 22:6) superior to 18:3n-3 [and] superior to 18:2n-6	Oka *et al.*, 1980
Turbot	19-20	11	0.6% n-3 superior to 3.7% 18:3n-3. Both are superior to 18:2n-6. 2-year old fish need 1% n-3	Leger *et al.*, 1979; Cowey *et al.*, 1976
	15	12		
Red sea bream	21-28	5-9	2% n-3	Fuji *et al.*, 1976
American lobster	20	40	n-3 rich in fish oil superior to 18:2n-6 rich in vegetable oil	Castell *et al.*, 1976; D'Abramo *et al.* 1980
Japanese prawn	24	7	1% 20:1n-3 or 22:6n-3 superior to 18:3n-3, 18:3n-3 superior to 18:2n-6	Kanazawa *et al.*, 1979(a,c)
	24	4		

Indian prawn	26	4	n-3 fish oil superior to 18:2n -6 or 18:3n-3 given alone. Adding 18:2n-6 and 18:3n-3 to fish oil superior to fish oil alone	Read, 1981
Red prawn	- - -	3	1% 18:3n-3 commercial edible oil	Showbart *et al.*, 1973
Long-legged prawn	17	6.5	need n-6 and n-6; optimum 18:2n-6/18:3n-3 ratio is 2:2; 20:5n-3 + 22:6n-3 superior to C18 PUFA	Martin, 1980
Crab	25	12	Fish oil superior to vegetable oil rich in 18:2n-6	Ponat *et al.*, 1980

Note: The table does not give the carbon number, indicating only n-3 or n-6: i.e., the n-3 series (Σn-3 PUFA) or n-6 series (Σn-6 PUFA).

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